Chapter 12
Antiviral Potency of Chitosan, Its Derivatives, and Nanocomposites

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Abstract Chitosan is a linear polysaccharide consisting of D-glucosamine and *N*-acetyl-D-glucosamine units. It is manufactured from chitin (a naturally abundant polysaccharide), extracted from the exoskeleton of crustaceans, squids, or fungi walls. Chitosan has a large number of amino groups making it positively charged moiety and, hence, is soluble in neutral as well as acidic solutions. It possesses a great deal of physical, chemical, and biological properties including biodegradability, biocompatibility, non-toxicity, display of antibacterial, antifungal and antiviral effects, and adsorption activity for heavy metal ions leading to its variety of applications in fields like water-waste management and treatment, cosmetic industry, food industry, drug and/or gene delivery, and wound healing and dressing. However, it has some shortcomings like high density because of extensive hydrogen bonding and high viscosity, hampering its activity. For this, many researchers over the years have functionalized chitosan and made use of nanoparticles resulting in chitosan nanocomposites, enhancing its properties. In this chapter, the focus would be on the noteworthy application of chitosan, modified chitosan, and its nanocomposites as viable antiviral agents against various animal and plant viruses. In fact, they have also been studied as a potential antiviral agent against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) which caused the Coronavirus Disease of 2019 (COVID-19) pandemic, which will also be discussed in detail.

Keywords Chitosan · Chitin · Coronavirus · COVID-19 · HIV · HPV · Plant virus

Abbreviations

ACE-2   Angiotensin converting enzyme-2

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AIDS	Acquired immune deficiency syndrome
AMV	Alfalfa mosaic virus
BYMV	Bean yellow mosaic virus
CMV	Cucumber mosaic virus
COVID-19	Coronavirus Disease of 2019
HA	Hemagglutinin
HCMV	Human cytomegalovirus
HCoV-NL63	Human coronavirus NL63
HCV-4a	Hepatitis C virus genotype 4a
HIV-1	Human immunodeficiency virus type-1
HPV	Human papillomavirus
HSV	Herpes simplex virus
HTCC	N-(2-hydroxypropyl)-3-trimethylammonium chitosan chloride
IL-10	Interleukin-10
MD	Molecular dynamics
NBCs	Nanobiocomposites
NO	Nitric oxide
PPE	Personal protective equipment
PSTV	Potato spindle tuber viroid
PSV	Peanut stunt virus
PVX	Potato virus X
RBD	Receptor binding domain
ROS	Reactive oxygen species
RSV	Respiratory syncytial virus
RVFV	Rift valley fever virus
SARS-Cov-2	Severe acute respiratory syndrome coronavirus-2
$TCID_{50}$	Tissue culture infectious dose
THI	T helper 1
TMV	Tobacco mosaic virus
TNV	Tobacco necrosis virus

1   Introduction

Chitosan (Fig. 1) is a polysaccharide and a co-polymer of β-(1 → 4)-linked D-glucosamines and N-acetyl–D-glucosamines. It is usually manufactured from the deacetylation of chitin, which is a homopolymer of β-(1 → 4) linkages of N-acetyl-glucosamine. Chitin is a natural polymer present in the exoskeletons of shrimp, shellfish and crabs, cuticles of some insects, and fungi, whereas chitosan is a man-made polymer. Chitosan has many free amino groups making it soluble in neutral as well as acidic solutions. Its solubility depends on the source of chitin, the degree of deacetylation of chitin, and its molecular weight [60]. The ease of production of

chitosan and the raw material used for its production makes it a low cost, environmentally benign, biodegradable, biocompatible, non-toxic biopolymer. Chitosan is a positively charged polysaccharide having a variety of applications in the field of pharmacy, medicine [60], tissue engineering [3], 3D bioprinting [32], dental specialties [82], drug delivery [56], and diagnosis of ailments, especially if coated by nanoparticles [77]. Apart from this, chitosan is an excellent antiviral [2] and antimicrobial agent [11, 62]. Chitosan and its derivatives have also been tested successfully against Coronavirus Disease of 2019 (COVID-19) [22]. Few other applications of chitosan are in wastewater treatment, air filtration, cosmetic industry, food industry, and drug delivery. But, chitosan has some drastic limitations related to its reactivity and processability. This could be owed to its high density because of the hydrogen bonding interactions between chains of chitosan and high viscosity because of the intramolecular electrostatic interactions. Such problems of chitosan could be overcome, without changing its original properties, by chemically modifying it and introducing functional groups via phosphorylation [70], quaternization [28], carboxyalkylation [75], and hydroxyalkylation [15], to name a few [20].

Chitosan exhibits antimicrobial activity against a broad range of plant and animal microbes [44, 63], but its insolubility in the aqueous medium hampers its antimicrobial activity. Hence, for enhancing its solubility, dispersity, and antimicrobial activity in agriculture, its solution is prepared in an acidic medium which induces toxicity [68]. Chitosan has been modified chemically using halogenated acetate, like N,N,N-trimethyl chitosan chloroacetate and N,N,N-trimethyl chitosan trifluoroacetate, which has been shown to exhibit higher solubility and, hence, higher antimicrobial activity as compared to the unmodified chitosan [84]. Another problem with chitosan is that it is easily degraded physically and biologically. The instability of chitosan in acidic and basic pH and solutions with different ionic strengths is another major cause of concern, hampering its activity. It gets dissolved in acidic pH owing to the presence of amino groups but precipitates out in basic pH due to the de-protonation of amino groups [50]. Hence, the only way out is to modify chitosan, enhancing its physicochemical properties without affecting its basic structure or bioactivity. One
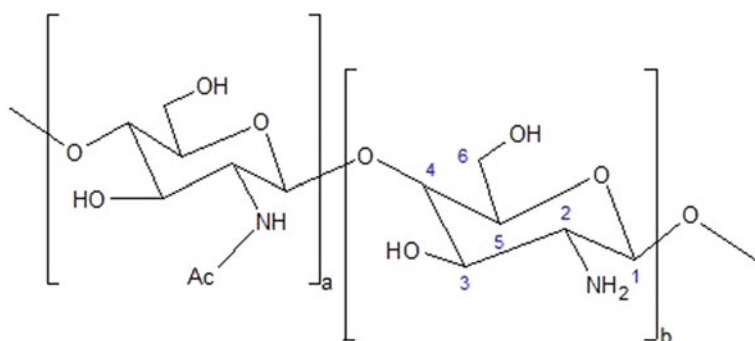

Fig. 1 Structure of chitosan

way of modifying it is by forming chelates with organic and inorganic compounds enhancing its stability, solubility, and biocidal activity [46, 67].

There is an array of factors that govern the antimicrobial activity of chitosan. Some of them include the type of synthesis process, conditions like temperature and pH, the extent of deacetylation from chitin, nature of substituents used to prepare modified chitosan, the molecular weight of chitosan, and/or its nanocomposites and derivatives as well as its charge, and also the source of chitin [29]. Chitosan, in itself, displays limited antiviral activity over a wide range of viruses, but modifications of the amino group and the hydroxyl group of chitosan have proved quite effective in improving its antiviral efficacy. For example, sulfated chitosan possesses better antiviral properties against human immunodeficiency virus type-1 (HIV-1) [71]. Since the antimicrobial activity of chitosan has vast applications against a lot of microbes, in this chapter, we have focused on and discussed the antiviral property of chitosan and its derivatives.

2 Use of Chitosan and Its Nanocomposites as Antiviral Agents

Chitosan and its composites are known to inhibit viral infection directly by damaging the viral cell membrane due to the electrostatic interaction of the positive charge of chitosan with negatively charged protein on the viral surface or/and by binding viral tail fibers. Chitosan can also inhibit viral infection indirectly by stimulating the immune response against viruses affecting plants, animals, and microorganisms. Chitosan and its derivatives also act as carriers of antiviral drugs (Fig. 2). A lot of factors influence the antiviral activity of chitosan, namely the extent of its polymerization and deacetylation, its charge, and the nature of the chemical changes. In fact, oligomers of chitosan were found to be more effective than their polymeric forms [13, 66].
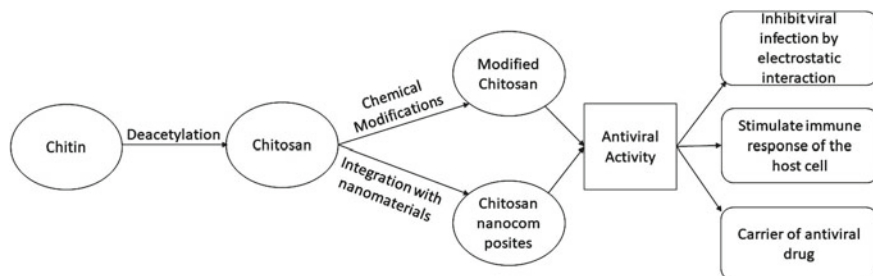

Fig. 2 Antiviral action of chitosan and its derivatives

2.1   Action of Chitosan, Its Derivatives, and Nanocomposites Against Animal Viruses

Acquired immune deficiency syndrome (AIDS) has been a major cause of concern over the years and is caused by HIV-1 retrovirus [18]. Many anti-HIV-1 agents are available, but their use is limited owing to many reasons like the emergence of mutated viruses resistant to such anti-HIV agents and their toxicity toward healthy cells [69]. Researchers have been constantly working toward developing new, alternative, and natural materials and their derivatives to improve the efficacy of drugs against HIV and reduce toxicity. Chitosan and its derivatives have emerged as a good candidate against HIV since they are biodegradable and non-toxic. N-carboxymethylchitosan N,O-sulfate was prepared and found to inhibit HIV-1 by inhibiting reverse transcriptase enzyme and hampering the binding of viral cells to human CD4$^+$ target cells (host cell receptor) [75]. Sulfated chitooligosaccharides with molecular weight in the range of 3–5 kDa, being non-toxic and water-soluble, were found to block entry of HIV and virus-cell fusion by hampering the binding of HIV-1 to CD4 cell surface receptor [7, 20]. Heparan sulfate binding receptor is the cell-binding receptor to which some viruses bind leading to their entry into the host cell. Such viruses are HIV-1, herpes simplex virus (HSV), human cytomegalovirus, and papillomaviruses [71]. Sulfate substituted succinyl chitosan was found to be effective against Moloney murine leukemia virus (having heparan sulfate binding receptor), as studied on infected rodent SC-1 and NIH-3T3 cell lines with the concentration of 0.01–100 µg mL^{-1}. This study revealed that high molecular weight and a high degree of substitution increase the antiviral efficacy of chitosan [76].

Chitosan oligomers having low molecular weight can be modified with peptides like tryptophan and glutamine and are found to have good anti-HIV-1 activity. This is achieved by the reduction in the amount of p24 protein (having an active role in maintaining the virus structure), envelope, and viral infection factor proteins on the viral surface, inhibiting the replication of the virus [41]. Another safe and non-toxic complex of chitosan, effective against HIV-1, is hyaluronan, stabilized with Zn(II) ions [83]. Chitosan complex with Ni(II) ions leads to an effective binding of Ni(II) with enterovirus-71 (a cause of infections in children and responsible for neurological diseases) [49, 71].

Human cytomegalovirus (HCMV) belongs to the betaherpesvirinae family of double-stranded DNA viruses with 55–100% infection in humans [65]. HCMV is asymptomatic in most healthy humans but could prove lethal for humans having weak immune responses, children, organ transplant recipients, and HIV-positive patients [24]. The available drugs like ganciclovir, cidofovir, and foscarnet [14] are toxic, have low bioavailability, and face resistance from the virus [79]. For developing a probable drug against HCMV, certain factors have to be accounted for, which have been integrated into a nanocomposite of ZnO nanoparticles with phenyloxy functionalized chitosan. Such nanocomposites exhibited excellent antiviral activity against HCMV in vitro with low cytotoxicity, which could be used as an independent drug or

as a combination with other available anti-HCMV drugs. The prepared nanocomposites of 4-hydroxybenzaldehyde, 4-(benzyloxy)benzaldehyde-modified chitosan with ZnO nanoparticles decreased the viral load interfering with the active replication of the virus [39].

A lot of research has been done by different research groups to study the antiviral property of chitosan and its derivatives. Chitosan displayed antiviral activity against adenovirus [59] and human noroviruses [16]. Silver nanoparticle–chitosan nanocomposite is quite effective against the H1N1 influenza A virus. Such a composite not only reduced the tissue culture infectious dose ($TCID_{50}$) ratio of virus suspension but reduced the toxicity as well by preventing the release of silver in the environment as it is fixed on chitosan [55]. The nanocomposite of chitosan with curcumin works against hepatitis C virus genotype 4a (HCV-4a) and prevents their entry and replication in the human hepatoma Huh7 cells [51].

Chitosan is known to enhance antiviral immune responses. They stimulate the innate immune cells by increasing the number of leukocytes and macrophages, reactive oxygen species (ROS), nitric oxide (NO), and enhancing the cell-mediated responses, to fight the pathogen. Macrophages can lead to phagocytosis of chitosan and, hence, leads to the production of ROS which induces the synthesis of gamma interferon in spleen cells, which in turn shows its antiviral activity by inhibiting the translation of RNAs of the viral cells [72].

A lot of studies were conducted on different murine models, which showed that the size of chitin particles is of immense importance in inducing the innate immune response of the host. Big-sized chitosan when enters the host, the immune response is stimulated which tends to break the chitosan into small parts with the help of chitinases. The intermediate-sized chitin particles send an alarm signal leading to the inflammation by activating the recognition receptors. This would continue until the virus infection is done with, and chitin is further oxidized into much smaller fragments. The small-sized chitin leads to the production of interleukin-10 (IL-10), which works toward controlling the inflammation. Similar response is by chitosan and mixture of chitin and chitosan [47]. Therefore, chitosan can induce innate immune responses against viral infections.

If vaccines are administered with chitin and chitosan-based nanoparticles, a drastic enhancement in the innate and adaptive immune response occurs. DNA vaccine in complexation with chitin microparticles was administered against HIV infection in BALB/c mice. Chitin was found to enhance the adaptive immune response as DNA/chitin microparticles were found to increase antibodies against HIV and their T cells [25]. Some other examples to illustrate this involve the administration of chitin along with chitosan, in BALB/c mice, for the inactivation of hemagglutinin (HA) protein (which mainly helps in binding host cells) of H1N1 influenza virus [26] and recombinant HA protein of H5N1 influenza virus [8]. Mucosal, as well as systemic humoral immune responses, was stimulated. Chitin along with chitosan was also found to increase the production of secretory IgA antibodies in the nasal wash and IgG antibodies in the serum causing T helper 1 (TH1) immune response against viruses [8, 26]. Chitin microparticles having a size of 10 μm, when administered in H5N1 and H1N1-infected mice through the intranasal route resulted in an increase in

the number of natural killer cells in the cervical lymph tissues. This led to a decrease in the viral load and enhanced survival rate after infection [31]. In another study, microparticles of chitin having a size of 3.72 μm enhanced the immune response of the host cell against the influenza A virus and also enhanced the production of T cells which helps in fighting against the virus [9].

Chitosan polymers could be used as a solution, in the form of powder, as microparticles, and nanoparticles, depending on their physical and chemical properties. They can also be used as carriers for antigen and/or adjuvants in mucosal vaccines as they can induce cellular and humoral immune responses. Certain examples of chitosan to illustrate this include its activity against the influenza virus. When chitosan is used as a solution (0.5%) along with inactivated influenza vaccines, the immunogenicity of the vaccine increases by six to ten times [23]. Again, chitosan solution when administered intranasally with influenza matrix protein induces and increases the amount of IgG and IgA antibodies [73, 74]. Chitosan solution has also been found effective with live attenuated influenza vaccine [81].

A range of chitosan derivatives like sialyl lactose substituted chitosan [48] and chitosan-sialyl oligosaccharides (obtained from bovine colostrum) complex have been found to be effective and selective against influenza virus A [12]. Such modified chitosans block the interaction of the host cell receptors with the viral surface by acting as virus adsorbents. This is because sialyl lactose and sialyl oligosaccharides are the receptors present in the host cells to which the virus binds and enter the host cells. By modifying the chitosan with such receptors present on the host cell, the virus gets adsorbed on the modified chitosan and, hence, is prevented from binding to the host cell receptors. Sulfated chitosan has proved to be quite effective against many widespread viral infections. For example, 3,6-*O*-sulfated chitosan with an average molecular weight of 58.3 kDa and having 45.8% sulfate content inhibits human papillomavirus (HPV), responsible for cervical cancer, by directly binding the viral proteins [21]. Apart from this, in vitro study in dunni cells of friend murine leukemia helper virus and HSV-1 revealed antiviral activity of sulfated carboxyl methyl chitin backbone (with 7.66% degree of sulfation) against these viruses [36]. Both these inhibitory activities of sulphated chitosan are dose dependent.

Chitosan nanoparticles made using *Lucilia cuprina* maggots showed effective antiviral activity in terms of the reduction in viral infection by 24.9% against Rift Valley fever virus (RVFV), 26.1% against Coxsackie viruses and 18.8% in HSV-1 on a Vero cell line of adult African green monkey kidney [27]. Noroviruses, usually present in contaminated food and water, could lead to gastroenteritis and diarrheal diseases if they enter the human body. Chitosan microparticles, yet again, could reduce the viral load in murine norovirus and bacteriophage MS2 of *Escherichia coli* at 0.3% concentration [71, 85].

Respiratory syncytial virus (RSV) affects the lower respiratory system and could be fatal for newborn children, elderly people, and people with low immune responses. Here again, the use of chitosan with vaccines made from RSV-coded DNA can increase the number of IgG antibodies in serum, IgA antibodies in mucous, and T cells which secrete interferon-γ and induces T lymphocyte response which could be

lethal for viral cells. This leads to a decrease in the number of viral cells, an increase in the antiviral response, and a reduction in the inflammation of the lungs [45, 66].

Among bacteriophage viruses, chitosan and its derivatives were studied against coliphages T2, T4, and T7 in *E. coli*. The degree of polymerization and functionalization, charge, and molecular weight of 6-*O*-sulfate and *N*-succinate-6-*O*-sulfate-modified chitosan has a profound effect on their activity against phage viruses. Studies revealed that the high degree of polymerization and high concentration of chitosan (100 and 10 µg mL^{-1}) have higher antiviral activity against phage viruses [42, 43, 71].

2.2 Action of Chitosan, Its Derivatives, and Nanocomposites Against Coronaviruses

COVID-19 pandemic has been widespread and has affected many countries around the world. It is a highly contagious disease and spreads quite fast through the air. The aerosol particles containing severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) viruses, present in the air via sneezing, breathing, coughing, and talking by a person carrying SARS-CoV-2 infection, could be inhaled by a healthy person in the vicinity leading to the spread of infection. Also, the virus particles may spread through the surfaces on which they settle [78]. The main problem with SARS-CoV-2 is that it has size and properties similar to a nanoparticle which is responsible for its high transmission rate. It possesses spike protein having polybasic arginine-rich motifs, making it more infectious than its previous SARS sequences [38, 66].

During this pandemic, frontline workers, including paramedics, doctors, and healthcare workers, who constantly come in touch with infected people on a daily basis, need to be protected. In this regard too, nanotechnology has come to the rescue. Chitosan nanoparticles being positively charged could be integrated into the clothes of frontline workers, which imparts electrostatic repulsion to the positively charged SARS-CoV-2 particles resulting in less viral load on and around them and, hence, the lower transmission of the virus. Chitosan nanofibers having a positive charge including single and double *N*-quaternized chitosan derivatives, like *N*,*N*,*N*-trimethyl chitosan, have been proposed to be useful in the production of personal protective equipment (PPE) for frontline workers, owing to their repulsive interactions with coronavirus [28].

Since the emergence of the COVID-19 pandemic, scientists around the globe have been in search of potent and non-toxic drugs against it since till now, no licensed drug is available against coronavirus. For coronavirus too, chitosan nanoparticles, its derivative, polymer, and nanocomposites could be effective, being biocompatible.

A large number of studies have been conducted and are still underway to prepare antiviral food coatings and fabric to prevent the spread and effect of coronavirus. In this regard too, chitosan has been proved quite useful. This inhibition of coronavirus by chitosan and their derivatives could be due to the electrostatic repulsive interaction

between the positively charged chitosan nanocomposite and the spike protein of coronaviruses [80]. A cross-linked derivative of chitosan with genipin (obtained from the plant), i.e., N-(2-hydroxypropyl)-3-trimethyl chitosan, was found to adsorb and inhibit the activity of human coronavirus NL63 (HCoV-NL63), reducing the infection potential of the virus by $(7.2 \pm 0.8) \times 106$ copies mL^{-1}. This was attributed to the electrostatic repulsive interaction between the positively charged chitosan derivative and the viral protein [15].

The addition of chitosan along with DNA, for example, plasmid DNA-loaded biotinylated chitosan nanoparticles, could enhance the immune response to a vaccine against SARS-CoV and could also reduce the side effects like inflammation of the lungs. A study was done by a group of researchers in 2012, [64], well before COVID-19 pandemic, wherein they studied the immune response of a DNA vaccine having chitosan nanoparticles against SARS-CoV nucleocapsid protein and found an increased number of IgG and IgA antibodies (an indicator of strong immune response of the host cell) against nucleocapsid protein, responsible for its enhanced immune response.

Chitosan has also been reported as an effective carrier for drugs. Apart from this, it has an ability to stimulate the immune system, is non-toxic, biocompatible, and degrades in the body releasing non-toxic by-products; it could easily open up tight intersections, and it could be modified into different shapes and sizes. All these useful properties make chitosan-based nanomaterials an effective tool to fight the COVID-19 pandemic [37]. Aerosol formulations of Novochizol™ nanoparticles, made of chitosan, were found to selectively deliver the potential anti-coronavirus drug to the lungs. When administered to a patient, Novochizol™ nanoparticles remain attached to the mucosal membranes of the respiratory tract. The chitosan molecule present is degraded slowly, releasing the active drug molecule in effective amounts [66].

Spike protein of SARS-CoV, SARS-CoV-2, and HCoV-NL63 (common cold virus) has receptor-binding domain (RBD) which binds the angiotensin-converting enzyme-2 (ACE-2) receptors of the human respiratory tract and enter the host cells causing infection. A chitosan-based polymer, N-(2-hydroxypropyl)-3-trimethylammonium chitosan chloride (HTCC), and its hydrophobic derivative were studied and found to exhibit inhibitory activity against not only HCoV-NL63 but against human murine hepatitis virus too. The main action of the polymer includes its inhibitory action for the interaction between the receptors of the host cell and the RBD of the viral spike protein. This could be achieved by aggregating spike proteins so that their interaction with the host cell reduces. The degree of substitution of chitosan in HTCC also had a varied effect on different human coronaviruses like HCoV-OC43, HCoV-229E, HCoV-NL63, and HCoV-HKU1 as revealed by the study conducted on LLC-MK2 (*Macaca mulatta* kidney epithelial) cell line [53, 54]. HTCC has also been an effective inhibitor of SARS-CoV-2 and Middle East respiratory syndrome coronavirus (MERS-CoV) [52], as demonstrated by in vitro studies conducted using Vero and Vero E6 cell lines and ex vivo studies using human airway epithelium mode, through the electrostatic interactions between HTCC and viruses. A molecular dynamics (MD) study also revealed the excellent potential of chitosan as a drug and in vaccines against coronavirus. It demonstrated a new target

on SARS-CoV-2 for chitosan apart from RBD, which is a homotrimer pocket of spike protein, which too has a strong affinity for ACE-2 receptors of the host cell [40]. β-chitosan was found to inhibit the binding of the spike protein of SARS-CoV-2 with ACE-2 receptors by itself binding with ACE-2. Experimental studies like in vitro and in vivo analysis and immunofluorescence demonstrated the downregulation of ACE-2 in Vero E6 cells of mice. A decrease in the immunofluorescence intensity from 33.6 to 6.91% was observed in the presence of β-chitosan. This has led to a drastic decrease in the colocalization of RBD and ACE-2 receptors, and thereby, their binding is inhibited [4, 71].

2.3 Action of Chitosan, Its Derivatives, and Nanocomposites Against Plant Viruses

Evidence is available for the inhibitory response of chitosan and its derivatives against plant viruses as well, but it needs to be explored more for their effective use in agriculture [17, 35]. Few studies showed the reduction of necrotic lesions caused by alfalfa mosaic virus (AMV), tobacco necrosis virus (TNV), tobacco mosaic virus (TMV), peanut stunt virus (PSV), cucumber mosaic virus (CMV), potato virus X (PVX), and potato spindle tuber viroid (PSTV) in *Phaseolus vulgaris*, *Pisum sativum*, *Nicotiana tabacum*, *Nicotiana glutinosa*, *Nicotiana paniculata*, *Lycopersicon esculentum*, *Chenopodium quinoa*, on spraying 0.1% chitosan [46, 61].

Although chitosan has remarkable antimicrobial properties owing to the electrostatic stacking of chitosan over the virus surface, the treatment of plants with chitosan increases the permeability of the membranes of plant cells disrupting their stability and, hence, cell death. However, in some viral infections, changes in the plant membrane could actually support their fight against the virus [6, 58].

Let us try to understand how chitosan derivatives fight against bean yellow mosaic virus (BYMV), responsible for mosaic and malformation of numerous plants. Safe and economical, carboxymethyl chitosan–titania nanobiocomposites (NBCs) were prepared and used to treat faba bean plants against BYMV. Here, *N*,*O*-substituted carboxymethyl (NBC1), and *O*-substituted carboxymethyl (NBC2) derivatives of chitosan, having low molecular weight, were synthesized. The severity of the disease was found to reduce drastically in the NBCs-treated faba beans by 10.66% and 19.33%, when administered with NBC1 and NBC2, respectively. Further, NBCs improved a lot of growth factors in plants, increased the number of photosynthetic pigments and water content, enhanced the stability of plant cell membrane, and increased the quantity of enzymatic and non-enzymatic antioxidants and soluble protein. Not only this, it reduced various phenomena which could adversely impact plant health like the leakage of electrolytes, amount of hydrogen peroxide, and the peroxidation process of lipids. Apart from impacting a lot of internal processes to maintain the stability and physiology of plant cells, NBCs were able to strengthen the immune response and systematic resistance of faba bean plants against BYMV. NBC1

was found to be more effective against BYMV owing to its higher hydrophilicity, biocompatibility, and chelation capacity as compared to NBC2. The antiviral action of NBCs is due to its constituents; TiO_2 nanoparticles and carboxyalkyl substituted chitosan, which provides protection against viral infection [74].

If one talks about the antiviral activity of chitosan in plants, they not only prevent the spread of viral infection but stimulate the resistance genes and a range of defense responses, as well, in plants. Here again, a range of factors including concentration, time of exposure, and poly-cationic nature of chitosan play an important role against the viral infection. The inhibitory activity of chitosan against plant viruses was also found to depend on its structure as well as its molecular weight. Chitosan with high molecular weight in the range of 100–120 kDa exhibited better and enhanced antiviral activity than those with lower molecular weight in the range of 3–36 kDa, against PVX infection in potatoes [13, 35]. This correlation between the molecular weight and the antiviral activity of chitosan does not always hold good. Sometimes, chitosan, having low molecular weight, displays better antiviral activity than its high molecular weight counterparts. One example to illustrate this is the inhibition of TMV by the chitosan having low molecular weight. The method of formation of the chitosan, having low molecular weight, from its high molecular weight precursor was found to influence its antiviral activity. Also, the source of chitosan and even the process used to purify chitosan affect their activity. The chemical hydrolysis (using H_2O_2) of chitosan producing low molecular weight chitosan was found to exhibit much better antiviral properties as compared to those obtained from the enzymatic hydrolysis (using lysozyme) [17, 66].

Not only the chitosan but the type of plant being affected and many times, the type of virus affecting the plant plays a noteworthy role in the extent of the response for chitosan. It is a well-known fact that the activity of lectin proteins, present in plants, is enhanced under biotic stress like low temperature, wound stress, or osmotic stress or when a virus or bacteria attacks a signaling plant, to resist the foreign organism. Chitosan can help augment the lectin activity and also develop resistance, as has been seen in the chitosan-treated tobacco plant and potato tuber trying to fight against TMV [10]. Chitosan is supposedly present in the cell membrane of the pathogen. When a pathogen interacts with the plant, due to structural changes, the chitosan molecule is degraded, and the products are released to stimulate a defense response against a pathogen, as revealed by a study conducted on tobacco against TNV [19]. The treatment of plants by chitosan leads to the deposition of callose, oxidative bursts, and hypersensitive responses, triggering the defense system of the plant against viruses [34]. Abscisic acid also leads to callose accumulation, and here also, chitosan plays a significant role in the accumulation of abscisic acid. This increase in the concentration of callose reduces viral-induced lesion sites and, hence, inhibits the spread of viral infection [33]. Chitosan is also responsible for an increase in the number of proteolytic enzymes like RNAses resulting in thinning of the virions and, hence, reducing their binding capacity, as depicted by the study conducted on leaves of *Nicotiana tabacum* L. cv. Samsun against TMV [57].

Chitosan alone and in combination with glycine betaine has also been evaluated for imparting resistance against CMV in cucumber plant. Gene expression analysis

showed the enhancement of defense genes. Apart from this, there is an enhancement of leaf chlorophyll content, hormones like salicylic acid and jasmonic acid, osmoprotectants like soluble sugars and proline, both enzymatic antioxidants like superoxide dismutase and peroxidase, and non-enzymatic antioxidants like ascorbic acid and glutathione in plants, in the presence of chitosan. On the other hand, the amount of malondialdehyde (an indicator of oxidative stress) and abscisic acid (stress hormone) reduces significantly in the presence of chitosan. The treatment of plants with the combination of glycine betaine and chitosan led to far better results as compared to the one using only chitosan [73]. Chitosan-*N* induces resistance against papaya mosaic virus (which causes the distortion of papaya leaves) in papaya leaf (*Carica papaya L*) by altering the metabolic pathways involving the metabolism of starch and sucrose, synthesis of phenylpropane, and transduction of plant hormone signal [5]. Chitosan actually induces systemic resistance against plant viruses by altering a lot of signaling pathways.

The duration of exposure to chitosan derivatives also impacts their antiviral activity. For example, exposure to 2 mg L^{-1} of guanidinylated chitosan hydrochloride for a short span of time leads to direct damage to the virus [30]. Another example includes effective inhibition by a high dosage of chitosan with different strains of rhizobacteria, which actually promotes the plant growth, in a time-dependent manner, against papaya ringspot virus-W and tomato chlorotic spot virus which affects cucurbits and tomatoes [1, 71].

3   Conclusion

In this chapter, the antiviral activity of micro- and nano-structures of chitosan and its derivatives along with its nanocomposites have been examined. Detailed study of chitosan against a plethora of animal viruses like HIV, HSV, HPV, influenza virus, and plant viruses like TNV and TMV showed that chitosan takes a triple approach in terms of its inhibitory activity. The main inhibition by chitosan is associated with its ability to inhibit the binding of the viral proteins with the receptors of the host cells and also reduce the viral load by strong electrostatic repulsive interactions with the virus, chitosan being cationic in nature. Chitosan also has a tendency to stimulate the immune response of the healthy cells against foreign organisms like a virus. Lastly, chitosan and its derivatives also act as good, selective, and efficient carriers for antiviral drugs.

The activity of chitosan depends on a lot of factors like the degree of polymerization, degree of substitution, the process of deacetylation, source of chitin, molecular weight, concentration, pH, and also on the type of virus in question.

Owing to a number of physicochemical properties of chitosan like cost-effectiveness, environment friendliness, biodegradability, biocompatibility, and non-toxicity, studies have been done to employ it as an anti-SARS-CoV molecule not only as a drug but as a component of nanofiber for the PPE kits. But, as far as its

activity against COVID-19 is concerned, there is still a long way to go in terms of the applicability of chitosan-based materials and their mechanism of action.

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